REMARKS

Claims 1 and 10 are pending in this application after entry of this paper. Claims 2 -9 have been canceled without prejudice. Applicants reserve the right to prosecute subject matter of the canceled claims in one or more continuation, continuation-in-part, or divisional applications. Claims 1 and 10 have been amended. No new matter has been introduced with these amendments. Applicants respectfully request reconsideration in view of the claim amendments and the following remarks.

Response to 35 U.S.C. §112, Second Paragraph Rejection

Claims 1 and 10 remain rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicants regard as the invention. Specifically, the Examiner contends that claims 1 and 10 are indefinite because the phrase "quantifying the accumulation of polyphosphate having a mean value strand length equal to or less than 50 mer and produced by the transformant after the expression has been induced" allegedly does not make sense. The Examiner alleges that the objected phrase is intended to limit which polyphosphates are being quantified; however, the phrase does not clearly set forth the metes and bounds of the polyphosphates being quantified. The Examiner further contends that it cannot be determined how to distinguish the PPK made by the transformant after expression has been induced from the PPK made by the transformant prior to the induced expression. Applicants respectfully disagree with this rejection.

The applicants have, however, amended the claims in order to expedite

prosecution of the instant application and to address the Examiner's concerns. Support may be found throughout the instant specification. One skilled in the art having read the instant specification would understand that the claimed host is directed to an animal or yeast whose PHM4 gene is knocked out. The claimed animal host does not possess a PPK gene, and does not substantially express a PPK gene. The claimed yeast host also does not possess a PPK gene, however, the yeast host does accumulate polyphosphate by its PHM4 gene. Therefore, a yeast host whose PHM4 gene is knocked out does not accumulate polyphosphate.

As stated above and in the instant specification, it is apparent that polyphosphate can be quantified in an animal host after PPK gene expression has been induced. (*See* paragraph 19 of the published specification.) Furthermore, polyphosphate can be quantified in a yeast host whose PHM4 gene is knocked out after PHM4 gene expression has been induced.

The Examiner contends that "[i]t cannot be determined how to distinguish polyphosphates having a mean value strand length equal or less than 50 mer from those that do not" (Office Action dated September 28, 2006 - page 4). Because polyphosphates having a mean value strand length greater than a 50 mer, which are outside the detectable range by NMR using ^{31}P-NMR measurement, a polyphosphate having a mean value strand length equal to or less than a 50 mer is naturally distinguishable by this technique. (*See* paragraph 16 of the published specification.) The instant specification states that polyphosphates of shorter lengths, especially less than 50 mer, are more sensitive and therefore easily detectable using the ^{31}P-NMR

technique. (*Id.*) Additionally, the polymer sizes are distinguishable by staining the cell contents with toluidine blue after polyacrylamide gel-electrophoresis. (*Id.*)

The Examiner also contends that the metes and bounds of when NMR is "non-destructive" in claims 1 and 10 are unclear. The term "non-destructive quantification" is used as a well-established technical term. The applicants believe that one of ordinary skill in the art would understand this term as it is used in the specification, for example, at paragraphs 5 and 6 of the published specification, and the claims. Also attached hereto is an abstract (A.A. van Apeldoorn, et al. "Parallel high-resolution confocal Raman SEM analysis of inorganic and organic bone matrix constituents" *J.R. Soc. Interface*. 2(2):39-45, March 22, 2005) that provides exemplary support for the understanding in the art at the time the application was filed that non-destructive imaging or non-destructive testing, non-destructive evaluation, or non-destructive inspection is any method that does not destroy or damage the test object. Therefore, no further clarification is necessary as one skilled in the art would understand the meaning of "non-destructive imaging."

Claims 1 and 10 have been amended to more clearly define the claimed invention, and solely for the purpose of advancing the prosecution of the instant application with respect to the Examiner's contention that it is unclear how "preparing a real time one-dimentional [sic] NMR profile" (Office Action dated September 28, 2006 - page 5) correlates to "quantifying the accumulation of polyphosphate," and further contends that it is unclear if they are separate steps or if the "preparing" further limits

how the "quantifying" is performed. The applicants assert that to prepare a real time one-dimensional NMR profile is the simplest method of quantifying accumulation of polyphosphate in a cell without altering the cell, *i.e.*, non-destructive. That is, the step of "preparing a real time one-dimensional NMR profile" thereby quantifies the accumulation of polyphosphate.

Step 4 of claims 1 and 10 have been rejected for the phrase "without adding an exogenous substrate" as being indefinite. As apparent from the following description of the specification, the description "without adding an exogenous substrate" further distinguishes the invention from the prior art. Therefore, the applicants argue that the use of this phrase is not indefinite. The instant specification provides examples of exogenous substrates where "β-galactosidase and luciferase, which require exogenous substrates, or GFP, which is autofluorescent, have been developed as reporter genes." (*See* paragraph 3 of the published specification.) The Examiner further states that "the phrase appears to relate to how the expression of PPK is induced and not how PPK expression is quantified as claimed." Applicants respectfully assert that, since PPK does not need to be added with an extracellular (exogenous) substrate, the cells do not need to be destroyed in order to isolate the expressed PPK from the cells.

The Examiner contends that the "comparison does not teach when a substance promotes or inhibits expression of the target gene." The applicants respectfully disagree. The applicants assert that it is not necessary to show when and at which stage (transcription stage, translation state, etc.) a substance promotes or inhibits the expression of the target gene. The specification clearly shows that a substance promotes or inhibits expression of the target gene, thereby enabling one of ordinary skill

in the art to achieve the claimed invention. The exact mechanism of action is not required to clearly set froth the claimed invention.

Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. §112, second paragraph for the above reasons and claim amendments.

Response to 35 U.S.C. §112, First Paragraph Rejection

Claims 1 and 10 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The applicants respectfully disagree with this rejection.

Specifically, the Examiner contends that the phrase "target gene" is new matter that is not supported by the claims or specification as originally filed. Applicants respectfully point out that this phrase was presented in claim 9 as originally filed on June 23, 2005. Moreover, this phrase is used in paragraph 18 of the published specification and examples of such target genes are set forth.

The Examiner contends that the step of "'preparing a plasmid' encoding PPK 'connected in frame and downstream of the target gene'" (Office Action dated September 28, 2006 - page 5) is new matter. Applicants respectfully disagree. However, in order to expedite prosecution of the instant application and solely for the purpose of advancing the allowance of the claims, claims 1 and 10 have been amended to claim a step of preparing a plasmid encoding PHM4 and PPK genes that are placed downstream of a target gene. Support for this step of the claimed method can be found

in paragraphs 13 (figure 3), 16, 19, and 25 and in the abstract of the published specification.

The specification supports the step where the plasmid is introduced into a host cell, a tissue, or an organ, and selecting a transformant. Support for this step can be found in paragraph 25 and the abstract of the published specification. Paragraph 25 of the published specification also supports the step of culturing the selected transformant.

Paragraphs 19, 20 and 25 of the published specification support the step of quantifying the accumulation of polyphosphate.

The step of comparing the accumulation of polyphosphate also finds support in the instant specification. Paragraph 20 of the published specification contains such support.

Since each step of the claimed invention is supported by the instant specification and claims as originally filed, the applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. §112, first paragraph.

Response to 35 U.S.C. §102(b)

Applicants respectfully acknowledge the withdrawal of the rejection of claim 1 under 35 U.S.C. §102(b) as being anticipated by Walter (*PNAS* 97:5151-5155, 2000); the rejection of claim 1 under 35 U.S.C. §102(b) as being anticipated by Gropman (*Curr. Neurol. Neurosci. Rep.* 1:185-194, 2001); the rejection of claim 1 under 35 U.S.C. §102(b) as being anticipated by Ozawa (*Biosci, Biotech, Biochem* 65:185-189, 2001); and the rejection of claim 1 under 35 U.S.C. §102(b) as being anticipated by Koretscky (*Proceedings of the 4th Int. Soc. Magnetic. Resonance Med.*, 1996, pg. 69).

Sharfstein

Claim 1 remains rejected under 35 U.S.C. §102(b) as being anticipated by Sharfstein (*Ann. NY Acad. Sci.,* 745: 77-91, 1994). The applicants respectfully disagree with this rejection.

As an initial matter, and as the Examiner knows, MPEP §2131 states that in order for a reference to anticipate a claim under 35 U.S.C. §102, the reference must teach each and every element of the claim. Sharfstein, et al. describe that by shifting the phosphate level in the medium from the phosphate starvation condition to the phosphate surplus condition, the accumulation of polyphosphates in an *E. coli* cell increases, and the rapid increase of the activities of PPK gene product and the moderate decrease of the activities of PPX gene product are observed. Sharfstein does not describe that a host is limited to an animal or a yeast whose PHM4 gene is knocked out, as claimed in the instant application. Since Sharfstein does not teach each and every element of claim 1, Sharfstein does not anticipate claim 1 under 35 U.S.C. §102. For the above reasons, and in view of the claim amendments, the applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. §102(b) for the above reason.

Van Voorthuysen

Claim 1 remains rejected under 35 U.S.C. §102(b) as being anticipated by van Voorthuysen (*J. Biotech.,* 77: 65-80, 2000). The applicants respectfully disagree with this rejection.

van Voorthuysen, et al. teach that polyphosphate is produced in a plant as a result of PPK expression, but this is not an experiment conducted for the purpose of monitoring gene expression. While van Voorthuysen uses a vector to target gene expression and measure polyphosphate concentrations, this reference does not teach using a yeast or animal host. Since this essential element is claimed in both claims 1 and 10 and is not taught in van Voorthuysen, et al., this reference does not anticipate the claimed invention under 35 U.S.C. §102(b). The applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. §102(b) for the above reasons and in view of the claim amendments.

CONCLUSION

Based on the foregoing amendments and remarks, the applicants respectfully request reconsideration and withdrawal of the rejection of claims and allowance of this application.

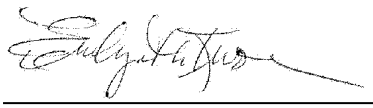
AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for consideration of this Amendment to Deposit Account No. **13-4500**, Order No. 4439-4023.

In the event that an extension of time is required, or which may be required in addition to that requested in a petition for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for an extension of time to Deposit Account No. **13-4500**, Order No. 4439-4023.

Respectfully submitted,
MORGAN & FINNEGAN, L.L.P.

Dated: December 28, 2006

By: 

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1: J R Soc Interface. 2005 Mar 22;2(2):39-45.

Parallel high-resolution confocal Raman SEM analysis of inorganic and organic bone matrix constituents.

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In many multi-disciplinary fields of science, such as tissue engineering, where material and biological sciences are combined, there is a need for a tool that combines ultrastructural and chemical data analysis in a non-destructive manner at high resolution. We show that a combination of confocal Raman spectroscopy (CRS) and scanning electron microscopy (SEM) can be used for such analysis. Studies of atomic composition can be done by X-ray microanalysis in SEM, but this is only possible for atomic numbers greater than five and does not reveal molecular identity. Raman spectroscopy, however, can provide information on molecular composition and identity by detection of wavelength shifts caused by molecular vibrations. In this study, CRS-SEM revealed that early in vitro-formed bone extracellular matrix (ECM) produced by rat osteoprogenitor cells resembles mature bone chemically. We gained insight into the structure and chemical composition of the ECM, which was composed of mainly mineralized collagen type I fibres and areas of dense carbonated calcium phosphate related to the collagen fibre density, as revealed by Raman imaging of SEM samples. We found that CRS-SEM allows the study of specimens in a non-destructive manner and provides high-resolution structural and chemical information about inorganic and organic constituents by parallel measurements on the same sample.

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